Comments on *In Vitro* ER / AR Assays

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In Vitro ER/AR Assays

- Public Law 106-545, Dec. 19, 2000 - any new or revised acute or chronic toxicity test method, including animal test methods and alternatives, must be determined to be valid for proposed use prior to an Agency requiring, recommending, or encouraging the application of such test method.
- Patent restriction issues (if applicable) must be addressed by EPA to maximize the potential use of these assays.

In Vitro ER/AR Assays

- Recombinant receptor proteins for binding assays should be promoted.
  - Reduce animal use
  - Efficiency
- Many options, *BUT*
  - altering assay parameters creates significant variability (Beresford et al., 2000; Charles et al., 2000).
  - inter-laboratory
    - variability
    - sensitivity
    - reproducibility and precision

In Vitro ER/AR Assays

- Performance-based criteria are insufficient for purposes of standardizing EDSP *in vitro* assays.
- 'Gold Standard' assays are needed.
  - Single methodologies properly standardized and validated
  - Standard to compare other, alternative protocols
- An ICCVAM type process should be used.
  - several laboratories / identical protocols / assess reproducibility and accuracy by using an agreed set of reference chemicals with various physical-chemical properties

In Vitro ER/AR Assays

- Definite pass-fail criteria for each *in vitro* assay:
  - acceptable coefficients of variation (CoVs)
  - techniques for assessing cytotoxicity
  - defined acceptable levels of cytotoxicity
  - specified numbers of replicate data points per experiment
  - cutoffs for positive / negative response relative to defined controls
- Good Laboratory Practice (GLP) provisions of the USEPA, OECD and/or MAFF should be required.